Research article

Influence of Percolation Patterns on Copper Uptake, and Growth and Yield with Copper-polluted Stratified Paddy Fields

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Abstract Copper(Cu), arsenic, and cadmium are designated as specific substances of the Agricultural Land Soil Pollution Prevention Act in Japan. It has been known that high Cu concentrations in soil layers reduce rice crop production and therefore agricultural practices such as soil dressing have been applied to minimize damage to crops by Cu pollution. In this study, we investigated the effects of percolation patterns of the plowsole and the subsoil on growth and yield, and Cu uptake of paddy rice. Four stratified paddy field models were constructed to conduct growth tests under the condition that the percolation patterns of plowsole and subsoil were in an open or closed system. These models had a plow layer and an upper plowsole made with 12.5cm-thickness of non-polluted soil dressing (3.7 mg/kg) and underlying 15cm-thickness of a polluted lower plowsole and a subsoil layer whose Cu concentrations were either higher (approximately 250 mg/kg) or lower (approximately 70 mg/kg) than Japanese safety standards (125 mg/kg). During the tests, a constant water-ponding system was adopted, and mid-summer drainage was not done. As a result, Cu concentrations in the rice grains were 5% significantly higher in the open system percolation models regardless of the original amount of Cu in the plowsole and subsoil. On the other hand, we did not recognize the significant difference in growth and yield of rice plants among the models. We concluded that the Cu concentrations in rice plants were affected by percolation patterns of the polluted plowsole and subsoil even though they were covered with non-polluted soil dressing layers.

Keywords copper, rice, percolation patterns, soil dressing

INTRODUCTION

Copper (Cu), Cadmium (Cd) and Arsenic are recognized as the specific contaminant heavy metals for agricultural lands and thus a variety of techniques for minimizing the heavy metals uptake of crops have been developed, for example, soil dressing, chemical measurement, phytoremediation, and breed improvement of rice plants. It has also been recommended to keep the soil in reduction condition by flooding during the whole growing period in order to reduce Cd and Cu uptake (Yamane et al., 1997; Asami, 2005; Inahara et al., 2007; Akahane et al., 2013).

The studies of Cu pollution in agricultural lands have mainly been focused on damage to crops such as growth inhibition, while Cd concentrations in brown rice itself have been another significant issue of soil pollution. The problem of soil Cu pollution has been of relatively small interest compared to Cd pollution. It is probably because Cu pollution rarely directly affects human health (Kobayashi, 1978, Takaishi et al., 2015) and the area affected by Cu pollution is not as large as that of Cd contamination in Japan. It is reported that in apple orchards, Bordeaux mixtures, mixture of copper sulphate and calcium carbonate have been used for a long time and thus soil Cu concentrations in some orchards are as high as several hundred mg/kg (dry soil) (Aoyama, 2009) while the safety standard of soil Cu concentrations in Japan is 125 mg/kg. Since apple farming is really hard work, elderly farmers, especially, tend to abandon their orchards. Some of the apple orchards in lowland had once been converted from paddy fields and there is a possibility that they will be restored to paddy fields, which require less labor. Therefore, it should be necessary and important to develop the technique of minimizing Cu uptake of paddy rice plants.

In Japan, soil dressing has mainly been applied for remediation of Cu polluted soil (Asami, 2010). Recently, Paul et al. (2011a, b) and Sasaki et al. (2016a, b) clarified that variations of percolation patterns of the plowsole and the subsoil using stratified paddy field models with soil dressing layers resulted in significant differences in Cd concentrations in the brown rice. Paul et al. (2011b) also showed that the percolation patterns affected the amount of Cu accumulation in rice plants even though they used non-polluted soil (12.2 mg/kg). Since the solubility of Cu increases under the oxidation condition and decreases in the reduction condition as in the case of Cd (Dong et al., 2007), the percolation patterns in stratified paddy fields may affect the Cu uptake and growth and yields of rice plants.

It has been reported that Cu polluted soil is likely to induce a Cu accumulation in the roots of paddy rice and a decrease in the number of panicles and the ratio of ripening (Chino et al., 1966; Shibuya, 1979). Shibuya (1979) also mentioned that the yields of brown rice had decreased by approximately 10% under the condition that Cu concentrations in the subsoil layer were higher than 200 mg/kg with a 15cm-thick soil dressing. These studies, however, did not consider utilizing the percolation patterns.

From the above, the objective of this study is to clarify whether percolation patterns affect the growth, the yields, and the Cu uptake of rice plants under the conditions that Cu concentrations are either under or above the Japanese safety standard (125 mg/kg). We prepared stratified paddy field models with approximately 70 mg/kg- and 250 mg/kg- Cu contaminated soil. The results were that the percolation patterns significantly changed Cu concentrations in the brown rice but neither the growth nor the yields.

METHODOLOGY

Table 1 shows the physical and chemical properties of the soils used in this study. Kanagi soil (Loam), 3.7 mg/kg Cu concentration, was sampled from a plow layer of the paddy field in Kanagi farm of Hirosaki University, Aomori prefecture. Bunkyou soil (Clay Loam) was made by adding a solution of $CuCl_2 \cdot 2H_2O$ to the soil which had been sampled from a plow layer of the paddy field on Bunkyo campus of Hirosaki University, Aomori prefecture, and both were mixed well. Cu concentrations in the soil of Bunkyo campus were originally 10.5 mg/kg. We produced two levels of Cu contaminated soil, Cu concentrations in which were either lower (71 mg/kg) or higher (247 mg/kg) than Japanese safety standard (125 mg/kg). These values are 15 and 50 times, respectively, as large as the average Cu concentration of non-contaminated paddy fields in Japan (4.47 mg/kg) (Asami, 2010). The organic matter content (OM) of the Kanagi soil and Bunkyou soil was 4.7% and 6.6%, respectively. The gravel, which contained 0.8 mg/kg, was used for the lower layer of the models since they were designed after the fashion of paddy fields near a river.

Table 1 Physical and chemical properties of soil samples and gravel

	Density	Soil Texture	MgO	CaO	K_2O	Cu	T-C	T-N	C/N	OM
	(g/cm^3)			(mg/kg)			(%)	(%)		(%)
Kanagi Soil	2.58	L	229	531	306	3.7	2.74	0.18	15.40	4.7
Bunkyou Soil	2.61	CL	219	1848	373	10.5	3.84	0.26	14.50	6.6
Gravel	2.68	-	-	-	-	0.8	0.04	0.01	4.00	0.1

Experimental Design

According to the previous report (Sasaki et al., 2016a), two types of stratified paddy field models were used for the experiment: the open-system percolation model and the closed-system percolation model. The percolation patterns were determined by Sasaki et al. (1992). Each stratified paddy field model was constructed in an iron box (30 cm×50 cm×70 cm) filled with three layers of soil. The plow layer was from 0cm to 10cm deep with non-polluted Kanagi soil (dry density in puddling condition was 1.04 g/cm^3). The plowsole was from 10cm to 20cm deep with non-polluted and polluted soil (dry density at the depth from 10cm to 12.5cm [non-polluted Kanagi soil] and from 12.5cm to 20cm [Cu mixed Bunkyou soil] were 1.23 g/cm^3 and 0.75 g/cm^3, respectively). The subsoil was from 20cm to 55cm deep with polluted Bunkyou soil and non-polluted gravel (dry density at the depth from 20cm to 27.5cm [polluted Bunkyou soil] and from 27.5cm to 55cm [the gravel] was 0.75 g/cm^3 and 1.40 g/cm^3, respectively). Those layers were formed by compaction. The authors defined O-70 and C-70 as the setting value of 71 mg/kg of the stratified paddy field modes. Similarly, O-250 and C-250 were defined as the setting value of 247 mg/kg of Cu concentrations ('O' and 'C' stand for the open-system and the closed-system percolation, respectively). The ground water levels of the open-system and the closed-system percolation models were controlled at 57.5cm and 12.5-20cm depth, respectively. In the closed-system percolation models, the holes in the side walls of iron box were blocked in order to prevent the penetration of the atmosphere. On the other hand, in the open-system percolation models, the holes in the side walls of the iron box were open in the lower part of the plowsole and the upper part of the subsoil in order to aerate those layers.

After the two types of models were prepared, fifteen paddy seedlings (the plant length and the leaf stage were from 12.5 to 17.5 cm and from 4.4 to 5.0 leaves, respectively) named '*Oryza sativa* L., Tsugaru Roman' were transplanted. The paddy seedlings were transplanted by 10cm intervals. As for fertilizer, 2g of N, 2g of P_2O_5 and 2g of K_2O were administered per model and mixed with the whole plow layer before transplanting. During the cultivation period, the water ponding condition was constantly adopted but the mid-summer drainage was not done. Transplanting of the paddy seedlings and harvesting were conducted at the end of May and at the end of September, respectively. The experiment with the stratified paddy field models was conducted in a greenhouse on the university campus.

Measuring Method

The examination of rice plants such as plant length, leaf stage, the number of stems and panicles, the weight of straw, the number of grains of brown rice and the weight of brown rice was done by the standard method of Iwate Agricultural Experimental Station (1981). The quantitative analysis of Cu concentrations in leaves, root, brown rice and soils extracted by HCl solution was carried out with atomic absorption spectroscopy (MAFF, 1979). Other measurements were also conducted in standard methods used in Japan. The Oxidation-Reduction Potential (ORP) meter (Central Kagaku Co., Ltd., model UC-203) was used for measuring oxidation-reduction potential (Eh). The ORP sensors were installed at depths of 5, 15, 20, 27.5, 37.5 and 47.5 cm of each model.

RESULTS AND DISCUSSION

Oxidation-reduction Potential (Eh)

The temporal changes of Eh are shown in Figs. 1~4. The plow layer of O-70 and O-250 became reduction layers (under -100 mV) while the plowsole and the subsoil became oxidation layers (over 400 mV). On the other hand, Eh values measured at the depths of C-70 and C-250 were gradually decreased after transplanting, and in due time all the layers became reduction layers as Eh values showed under 0 mV. This means that, in this study, the polluted soil layers in O-70 and -250 were under oxidation condition while those layers in C-70 and C-250 were under reduction condition. It has been pointed out that the Cu uptake in rice is affected by the oxidation-reduction environment (Matsunaka, 2014) and, therefore, in this study, Cu solubility was probably high in the models of O-70 and O-250 (Takaishi et al., 2015). We decided on the oxidation and reduction condition on the basis of Yamane (1982), who had defined the oxidation layer as Eh value as 300 mV or more and reduction layer as < 300 mV.

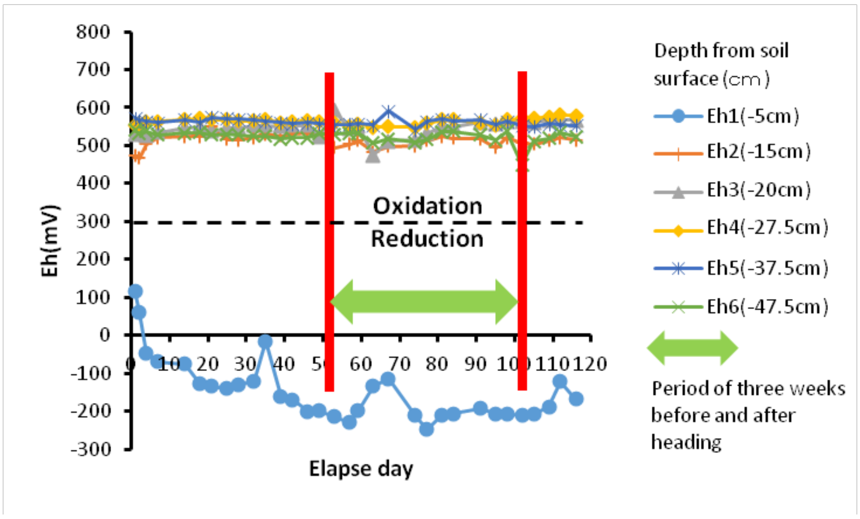

Fig.1 The temporal changes of Eh in the stratified paddy field model (O-70)

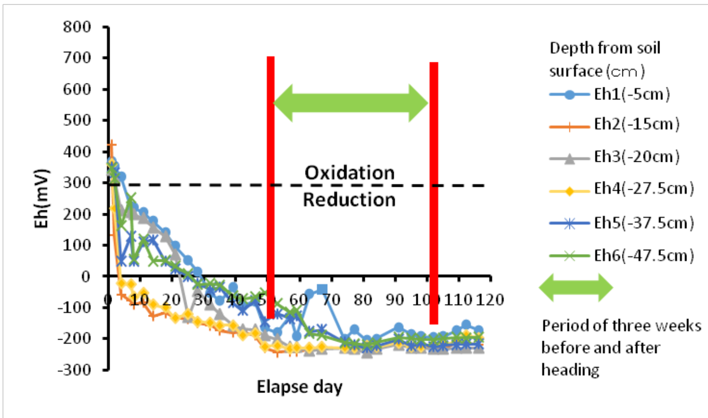

Fig.2 The temporal changes of Eh in the stratified paddy field model (C-70)

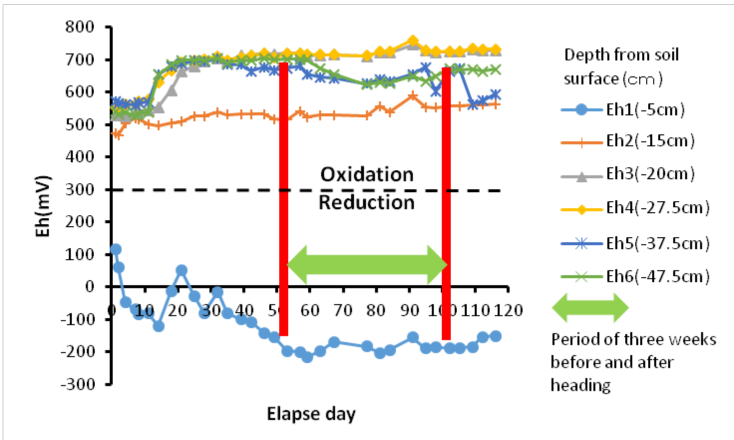

Fig.3 The temporal changes of Eh in the stratified paddy field model (O-250)

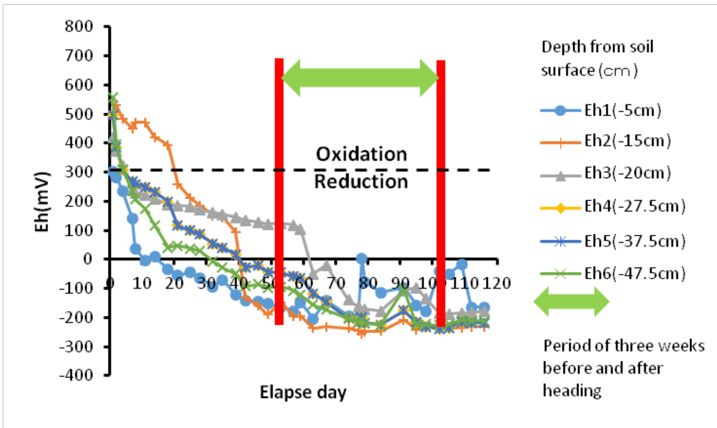

Fig.4 The temporal changes of Eh in the stratified paddy field model (C-250)

Copper Concentrations in Rice Plants

The results of Cu concentrations in rice plants are listed in Table 2.

Rice grains: Cu concentrations in brown rice ranged from 2.8 to 4.3 mg/kg which were similar to the values reported by Asami (2010). Their studies, however, did not make a distinction between open and close percolation patterns. Paul et al. (2011b) reported that the significant difference was observed in Cu concentrations in brown rice due to differences in percolation patterns. In addition, in their study, despite the low soil Cu concentrations, Cu concentrations in brown rice ranged from 2.5 to 4.2 mg/kg, similar to the results of our experiment. Thus the Cu concentrations in the lower layer did not make much difference in Cu concentrations in brown rice. From these results, it was revealed that the differences in Cu concentrations in brown rice due to differences in percolation patterns were confirmed as those in Cd concentrations found in the experiments conducted by Sasaki et al. (2016a, b).

Stems and leaves: It confirmed that statistically significant differences in the Cu concentrations in stems and leaves were between O-250 (1.6 mg/kg) and C-250 (0.8 mg/kg). However, there was no significant difference between O-70 and C-70. This discrepancy might be caused by the soil Cu concentrations, but this requires further elucidation.

Roots: In Cu concentrations in roots, significant differences in the high concentration models of O-250 (20.3 mg/kg) and C-250 (13.7 mg/kg) were recognized. However, significant differences in the low Cu concentration models due to the percolation patterns were not confirmed. Here, further discussion is needed to clarify its mechanism as in the case of stems and leaves.

Cu concentrations in the rice plants were in the order of roots > brown rice > stems and leaves. This order was similar to those of Shibuya (1979) and Paul et al. (2011b) who used rice plants, and to those of Li et al. (2017) who used soybeans. This result is probably due to the transport characteristics of Cu and Cd in the rice plants.

Table 2 Cu concentrations in rice grains, stems and leaves and roots in plow layer (mg/kg)

Model	Rice grains n=10	Stem and leaves n=5	Root of plow layer n=8
O-70	4.3±0.3[a]	1.5±0.4[a]	16.0±2.3[a]
C-70	3.4±0.4[b]	1.2±0.4[ab]	17.8±2.7[a]
O-250	4.1±0.4[a]	1.6±0.2[a]	20.3±1.4[b]
C-250	2.8±0.2[c]	0.8±0.1[b]	13.7±0.5[a]

Growth and Yield of Rice Plants

Experimental results for the growth and yield of rice plants are shown in Tables 3 and 4, respectively.

Growth of rice plants: The average plant height (n = 10) of each model was almost equal, at 90 cm level (Table 3). Leaf age of each model was about 14, showing a small difference between them. Total straw weight was 12.3～15.0 g/hill. No significant difference was observed in the plant height, leaf age and total straw weight regardless of the percolation patterns. Previous research (Shibuya, 1979) reported that Cu concentrations in the Cu polluted soil layer had an influence on the growth of rice plants. In this study, however, influence of the Cu concentrations on the growth of rice plants was not noticeable, which may well to have resulted from the application of soil dressing.

Yield of rice plants: An average number of panicles per unit hill in each model was 8.6～9.6 /hill (Table 4). Likewise, averages of the weight of one panicle and the number of grains of brown rice per unit hill were 1.9～2.2 g / panicle and 616～680 grains /hill, respectively. In addition, averages of the percentage of ripening and the 1,000 grain weight of brown rice were 86.5～93.7% and 19.1～19.9 g, respectively. No significant differences were found in any of the items of the models with different percolation types. Paul et al. (2011a) reported that yield components of the closed-system percolation model were significantly higher than those of the open-system percolation model though their experiment was conducted by using a different soil type for Cd polluted soil layers. Sasaki et al. (2016a) conducted an experiment using stratified paddy field models with a Cd polluted soil layer and reported that there was no significant difference between the models with different percolation patterns. Soil dressing was presumed to be one of the reasons why no significant difference was observed in that study. In a previous study (Shibuya, 1979), it was reported that Cu concentrations had an influence on the number of panicles and the percentage of ripening. In this study, however, the influence of the Cu concentrations on the growth of rice plants was not remarkable, which may be attributed to the soil dressing.

Table 3 Parameters of rice plant growth

Model	Plant length (cm)	Leaf age (leaf)	Weight of dry straw (g/hill)
O-70	99.6±5.1[a]	14.3±0.5[a]	13.3±2.4[a]
C-70	99.6±3.8[a]	14.3±0.5[a]	15.0±2.2[a]
O-250	95.6±4.4[a]	14.0±0.0[a]	13.3±3.0[a]
C-250	99.7±5.5[a]	14.3±0.5[a]	12.3±1.8[a]

Note: Tukey-Kramer test was performed at 5% level; letter indicates significant difference. The numerical value of ± shows standard deviation

Table 4 Parameters of rice plant yield

Model	Weight of one panicle (g)	No. of Panicles (Panicles/hill)	Percentage of ripening (%)	Number of brown rice per unit hill (grains/hill)	1000 grain weight of brown rice (g)
O-70	1.9±0.3[a]	9.4±1.1[a]	92.3±2.2[a]	644.3±116.0[a]	19.1±0.9[a]
C-70	2.0±0.2[a]	9.6±1.2[a]	86.5±4.1[b]	616.9±118.8[a]	19.7±0.5[a]
O-250	2.2±0.3[a]	8.6±1.3[a]	93.1±1.5[a]	680.5±163.9[a]	19.9±0.8[a]
C-250	2.0±0.2[a]	9.6±1.8[a]	93.7±1.5[a]	627.9±65.0[a]	19.4±0.8[a]

Note: Tukey-Kramer test was performed at 5% level; letter indicates significant difference.
The numerical value of ± shows standard deviation

CONCLUSION

Using four types of Cu polluted stratified paddy field models, we conducted an experiment to clarify the effects of percolation patterns in the sub-layer (both plowsole and subsoil) on the Cu concentrations in the rice plants and their growth and yield. The models had a 15-cm thick Cu polluted soil layer and a 12.5-cm thick non-polluted soil dressing. For the Cu polluted soil layer, two different Cu concentrations of 71 mg/kg and 247 mg/kg were prepared.

The results of our experiment showed that in the open system percolation models the sub-layers became oxidation layers and those in the closed system percolation models the sub-layers became reduction layers. Cu concentrations in the brown rice of the open system percolation models were significantly larger by 5% than those of the closed system percolation models. In the models with concentrations of Cu (247 mg/kg), the Cu concentrations in stems and leaves and roots showed significantly different values between the percolation patterns. However, there was no statistically significant difference in the growth and yield of rice plants between the percolation patterns.

Under the above conditions, difference in percolation patterns of the stratified paddy field models did not affect the growth and yield of rice plants, while it had an influence on the Cu concentration in the rice plants.

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